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Two distinct mechanisms regulate luteovirus transmission efficiency and specificity at the aphid salivary gland

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Barley yellow dwarf luteovirus (BYDV) particles are transmitted by aphids in a species-specific manner. Transmission to plants requires that the virus particles be transported across the basal lamina and plasmalemma of the accessory salivary gland (ASG). To characterize the role of the ASG basal lamina in regulating BYDV transmission, five aphid species were microinjected with purified New York isolates BYDV-PAV or -RPV. Both viruses associated specifically only with the ASG basal lamina. The ability of virions to penetrate the basal lamina was separate from the ability to penetrate the plasmalemma. When the salivary glands of vector, *Sitobion avenae*, or non-vector, *Rhopalosiphum maidis*, aphids were incubated *in vitro* with New York isolate BYDV-MAV, virions only attached to the ASG basal lamina of *S. avenae*. When anionic and cationic ferritin were microinjected into aphids, only cationic ferritin aggregated on the surface of the ASG basal lamina and at openings of plasmalemma invaginations into the cytoplasm, suggesting that these sites had a net negative charge. *In vitro* studies of anionic and cationic gold penetration of ASG basal laminae indicated a macromolecular size exclusion limit of approximately 20 nm that depended on charge. Anionic gold particles did not accumulate in the basal lamina as densely as the 25 nm BYDV particles, suggesting that the virus particles have a greater affinity for the ASG basal lamina. These results indicate that both the ASG basal lamina and plasmalemma contain specific components independently involved in the recognition and transmission of luteoviruses.

Introduction

Luteoviruses are transmitted by aphids in a circulative, non-propagative manner (Gildow, 1987). The ingested virus is actively transported by receptor-mediated endocytosis across gut epithelial cells and released into the haemocoel of the aphid (Gildow, 1993). Virus is transmitted only if virions traverse the extracellular basal lamina (basement membrane) (Alberts *et al.*, 1989) surrounding the accessory salivary gland (ASG) and are transported through the underlying plasmalemma into the salivary canal. Transport of luteoviruses across the ASG basal lamina and basal plasmalemma can be luteovirus- or aphid species-specific events (Gildow & Gray, 1993).

Basal laminae are complex, multifunctional structures surrounding most animal tissues (Alberts *et al.*, 1989). They function in cell attachment and support, and they may serve as molecular sieves. The mechanism(s) for macromolecule transport through basal laminae are unknown, but may involve the disassembly and reassembly of the heteropolymers that form the sieve-like structure of the membrane (Yerchenco & Schittny, 1990). The main components of basal laminae are laminin, collagen type IV and fibronectin. Several other glycoproteins and proteoglycans may be present and contribute to the heterogeneity among basal laminae associated with different tissue types (Pedersen, 1991).

Studies of the New York MAV isolate of barley yellow dwarf virus (BYDV-MAV) indicated that the basal lamina surrounding the ASG of aphids is a potential selective barrier to the transmission of MAV (Gildow & Gray, 1993). Few virions of MAV were able to penetrate the salivary gland basal laminae of poor or non-vector aphid species. In addition, pre-incubating the virus with anti-capsid monoclonal antibody Fab fragments prior to injecting into vector aphids prevented attachment to the ASG basal lamina. These observations suggested that specific attachment sites on the ASG basal

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lamina recognize luteoviruses. Attachment may involve a receptor similar to the high-affinity laminin receptor associated with the basal lamina of mosquito cells and used by Sindbis virus (Wang *et al*., 1992). Surface carbohydrates on the basal laminae of insect salivary glands have also been implicated as potential surface receptors for trypanosomes and sporozoites (Perrone *et al*., 1986; Olson *et al*., 1990).

This study had three objectives. First, to determine whether the ability of the basal lamina to regulate virus penetration into the ASG is a general mechanism regulating luteovirus transmission, we compared the ability of the structurally distinct New York isolates BYDV-RPV and BYDV-PAV to associate with the basal laminae of vector or non-vector aphid species. Second, we studied the ability of the ASG basal lamina to bind luteoviruses *in vitro*, allowing the development of an affinity binding assay for future studies of virus protein–basal lamina interactions. Third, we tested the ability of macromolecules to penetrate ASG basal laminae to determine the size exclusion limits of the molecular sieving activity and to determine whether virus penetration of the basal lamina is an active process requiring modification of the basal lamina structure.

Methods

■ **Aphid colony maintenance and transmission tests.** Virus-free colonies of the New York biotypes of *Rhopalosiphum padi* (L.), *Rhopalosiphum maidis* (Fitch), *Sitobion avenae* (Fabricus), and *Schizaphis graminum* (Rondani) (Rochow, 1969) and two biotypes of *Metopolophium dirhodum* (Walker) were maintained as previously described (Rochow, 1969). The *M. dirhodum* biotypes originated from the same California clone (Gildow & Rochow, 1983) and have been reared in a similar manner at separate sites for 10 years at Cornell University, NY, USA (*M. dirhodum*-CU), and at Pennsylvania State University, Pa, USA (*M. dirhodum*-PSU).

The New York BYDV isolate designated RPV was transmitted most efficiently by *R. padi*; PAV was transmitted by *R. padi* and *S. avenae*; and MAV was transmitted most efficiently by *S. avenae* (Rochow, 1969). All were maintained separately in infected oats (*Avena sativa* L. cv. 'Coast Black') in a greenhouse under natural light. To infect oats, virus-free aphids were given a 48 h acquisition access period on detached leaves of BYDV-infected oats. Aphids were then transferred to 1-week-old oat seedlings. Five to ten aphids were caged on each seedling and given a 72 h inoculation access period at 20 °C under constant light. The plants were then fumigated with DDVP [*O*,*O*-dimethyl-*O*-(2,2-dichlorovinyl) phosphate] to kill the aphids and maintained in the greenhouse. Infected leaf tissue was harvested 3–4 weeks after inoculation and stored at −80 °C. Virus was purified as described by Webby & Lister (1992).

■ **BYDV penetration of basal laminae.** To study the interaction between BYDV isolates and the aphid ASG basal lamina, third or fourth instar, virus-free aphids were injected with purified virus diluted in 0·01 M phosphate buffer. Aphids were anaesthetized with CO_2, then injected dorsally at the thorax–abdomen junction with approximately 0·02 µl virus inoculum as described by Gildow & Gray (1993). Individual aphids were placed on 1-week-old oat seedlings and allowed a 24 h inoculation access period. After 24 h, aphids were removed and the seedlings were fumigated with DDVP, then maintained in the greenhouse and observed for BYDV symptom expression over a period of 4–6 weeks. Aphids which had been removed from the seedlings were prepared for transmission electron microscopy (TEM) and viewed as described previously (Gildow & Gray, 1993). The density of virions embedded in ASG basal laminae was determined by counting the number of virions in a 10 µm section of basal lamina at the anterior of the gland. A 10 µm length of basal lamina 75 nm wide and 70 nm thick would contain 0·05 μm^3 tissue. Particle density was calculated and averaged for six aphids in each aphid–virus isolate combination.

To study the interaction between a non-luteovirus and aphid ASGs, aphids were injected with 0·02 µl cowpea mosaic virus (CPMV), purified from cowpea (*Vigna unguiculata*) by polyethylene glycol precipitation and density gradient centrifugation. Injected aphids were fixed 24 h after injection and examined by TEM.

■ **Ferritin penetration of basal laminae.** To examine possible charge differences between *S. avenae* and *R. maidis* ASG basal laminae, aphids were microinjected with approximately 0·04 µl undiluted anionic (100 mg/ml, Miles Laboratories) or cationic (10 mg/ml, Electron Microscopy Sciences) ferritin. After injection, aphids were allowed to recover on damp filter paper in closed plastic dishes at 20 °C. Four to six hours after injection, aphids were fixed and prepared for TEM (Gildow & Gray, 1993).

■ **Colloidal gold.** Colloidal gold was used as an electron-dense particulate marker to study the effect of size and charge on the ability of particles to penetrate the ASG basal lamina. Unconjugated (anionic) 20 and 30 nm colloidal gold was purchased from EY Laboratories. Poly-L-lysine-conjugated (cationic) colloidal gold was purchased from Electron Microscopy Sciences. Seven to fourteen nm colloidal gold was prepared by the method described by Slot & Geuze (1985). Average particle diameters from preparations made with 5, 30 and 125 µl tannic acid were 13·8, 10·8 and 6·8 nm, respectively. Equal amounts of suspensions of colloidal gold of each size were combined and concentrated in Millipore Ultrafree-MC 10 000 NMWL Filters (Bedford). Gold was resuspended in one-hundredth of the original volume of 0·01 M phosphate buffer. Anionic 20 and 30 nm gold were concentrated in the same manner. Cationic 20 nm gold was not concentrated.

■ **Stability of colloidal gold *in vivo*.** To study the effect of aphid haemolymph on colloidal gold, adult *S. avenae* were injected with undiluted colloidal gold. Microinjection needles were used to withdraw haemolymph from aphids 5–30 min after injection. To prevent agglutination of haemolymph, the needles contained a small amount of aqueous 200 mM phenylthiocarbamide (PTU). Drops of haemolymph and PTU were expelled onto Parafilm and copper mesh grids coated with formvar and carbon were floated on the drops for 30 min. Grids were then stained with aqueous 2% uranyl acetate for 4 min and observed by transmission TEM.

To study the ability of gold particles to penetrate the basal lamina *in vivo*, adult *S. avenae* were injected with undiluted or concentrated colloidal gold and prepared for TEM as described above.

■ **Luteovirus and colloidal gold penetration of basal laminae *in vitro*.** Alate *S. avenae* were submerged in 0·1% glutaraldehyde in 0·05 M sodium cacodylate buffer. Fine point dissecting needles were used to hold the thorax and pull off the head. This procedure left the accessory and principal salivary glands dangling from the head capsule, which could be grasped with forceps for manipulation. Salivary glands and the attached head capsule were incubated for 15 min in 0·1% glutaraldehyde, rinsed in buffer and then incubated in virus (200 µg/ml)

or gold solutions for 1 h. Alternatively, salivary glands were dissected in 0·01 M phosphate buffer and immediately incubated in virus or gold. All incubations were done at 4 °C on a rotary shaker. Following incubation, glands were fixed in 1% glutaraldehyde, 2% formaldehyde in 0·05 M sodium cacodylate buffer and prepared for TEM.

Results

BYDV penetration of basal laminae

When five species of aphids were injected with the same preparation of purified RPV, three different types of interaction were observed between virions and the basal lamina surrounding the aphid ASG. In *R. padi*, *S. graminum* and *S. avenae*, virions were seen embedded throughout the basal lamina and adjacent to the basal plasmalemma within membrane invaginations, indicating that the virions had penetrated through the basal lamina (Fig. 1*a*). In *M. dirhodum*, most virions were observed embedded in the outer layer of the basal lamina exposed to the haemocoel (Fig. 1*b*). In *R. maidis*, RPV was not observed to associate with the ASG (Fig. 1*c*).

The vector, *R. padi*, transmitted RPV efficiently when injected with either 0·7 or 1·4 ng of virus (Table 1). Virions were consistently observed embedded in the basal lamina and intracellularly in clathrin-coated endocytotic and exocytotic vesicles, tubular vesicles and the salivary canal lumen. These membrane-bound organelles have been associated with luteovirus transport through the ASG (Gildow, 1987).

In the inefficient vector, *S. graminum*, virions of RPV occurred at low densities in ASG basal laminae (Table 1). When injected at 0·7 ng per aphid, RPV was not observed intracellularly in the ASG and no virus was transmitted. When injected at 1·4 ng, RPV was observed in endocytotic vesicles in 2 of 6 aphids examined and virus was transmitted by 3 of 20 aphids tested.

The non-vector, *S. avenae*, did not transmit RPV when injected with either 0·7 or 1·4 ng of virus. However, high densities of RPV virions were observed embedded in ASG basal laminae (Table 1) and appeared similar to those shown for *R. padi* in Fig. 1(*a*). When *S. avenae* was injected with 0·7 ng RPV, the density of virions embedded in the basal lamina was nearly three times that observed in the vector, *R. padi*. Although RPV accumulated to high levels and readily penetrated the basal lamina to accumulate adjacent to the plasmalemma, virions did not penetrate the plasmalemma, were never observed in salivary canals, and were not transmitted by *S. avenae*. In *S. avenae*, the ASG basal lamina is not a barrier to RPV and its inability to penetrate the ASG plasmalemma is the only determinant of vector specificity.

Two clones of the non-vector *M. dirhodum* (-CU and -PSU) were compared, with similar results. Virions of RPV injected at 0·7 and 1·4 ng per aphid were able to attach to and penetrate into the ASG basal lamina, but at much lower densities than in *R. padi* or *S. avenae* (Table 1). Most of the virions observed in the basal lamina were located in the outer half nearest the haemocoel (Fig. 1*b*). Virions were only rarely observed in the half of the basal lamina nearest the plasmalemma or in plasmalemma invaginations, suggesting that the virions rarely penetrated through the basal lamina. Virions were never observed in the salivary canals and RPV was not transmitted by the *M. dirhodum* tested in bioassays (Table 1).

In *R. maidis* injected with either 0·7 or 1·4 ng RPV, virions were occasionally observed in the haemocoel, but never associated with the ASG basal laminae (Fig. 1*c*). Virions were not observed intracellularly or in salivary canal lumens and RPV was not transmitted by *R. maidis* (Table 1).

When the NY-PAV isolate was injected into aphids at 1 ng per aphid, virions were observed embedded in the ASG basal laminae of *R. padi*, *S. graminum*, *S. avenae* and *M. dirhodum*-PSU, but not in *R. maidis* (Table 2). Although PAV virions were readily observed in the ASG basal laminae of vector species, the virus density per unit basal lamina was very low and transmission by *R. padi*, *S. graminum* and *S. avenae* was inefficient. *M. dirhodum* and *R. maidis* did not transmit when injected with 1 ng PAV. When the experiment was repeated at 200 µg/ml (4 ng per aphid), *R. padi*, *S. graminum*, *S. avenae* and *M. dirhodum* transmitted PAV, but *R. maidis* did not (Table 2).

The association of PAV with the aphid ASG basal laminae was similar to that previously shown for RPV in Fig. 1(*a*–*c*). Virions were observed to have penetrated the basal laminae of all four vector species. Virions also were observed intracellularly in the ASG and in the salivary canals. Virion density was highest in the most efficient vector, *R. padi*, and lowest in the least efficient vector, *S. graminum*. As for RPV, virions of PAV tended to accumulate in the basal laminae of *M. dirhodum* in the area adjacent to the haemocoel. Virions of PAV were not observed associated with the ASG basal lamina of *R. maidis*, and this species did not transmit the virus when injected with 4 ng of virus (Table 2).

Test for CPMV association with ASG basal laminae

To test if association of virus with the ASG basal lamina was a general phenomenon that might occur with any virus, *R. padi*, *S. avenae*, *M. dirhodum* and *R. maidis*, were injected with 20 ng CPMV per aphid and examined after 24 h feeding on oats. Virions of CPMV are similar in size and shape to BYDV, but are not aphid-transmitted. The ASGs of three aphids of each of four species were examined for association of CPMV with the basal laminae. In two replicate experiments, virions of CPMV were consistently observed suspended in haemolymph in the haemocoel near the salivary glands (data not shown). However, CPMV was never observed attached to, embedded in, or penetrating the basal laminae of any tissues examined in any of the four aphid species. In contrast to BYDV, CPMV did not appear to be concentrated at the ASG or to associate with any other tissues, including the principal salivary gland, suboesophageal nerve ganglion, stylet retractor muscle or connective tissue.

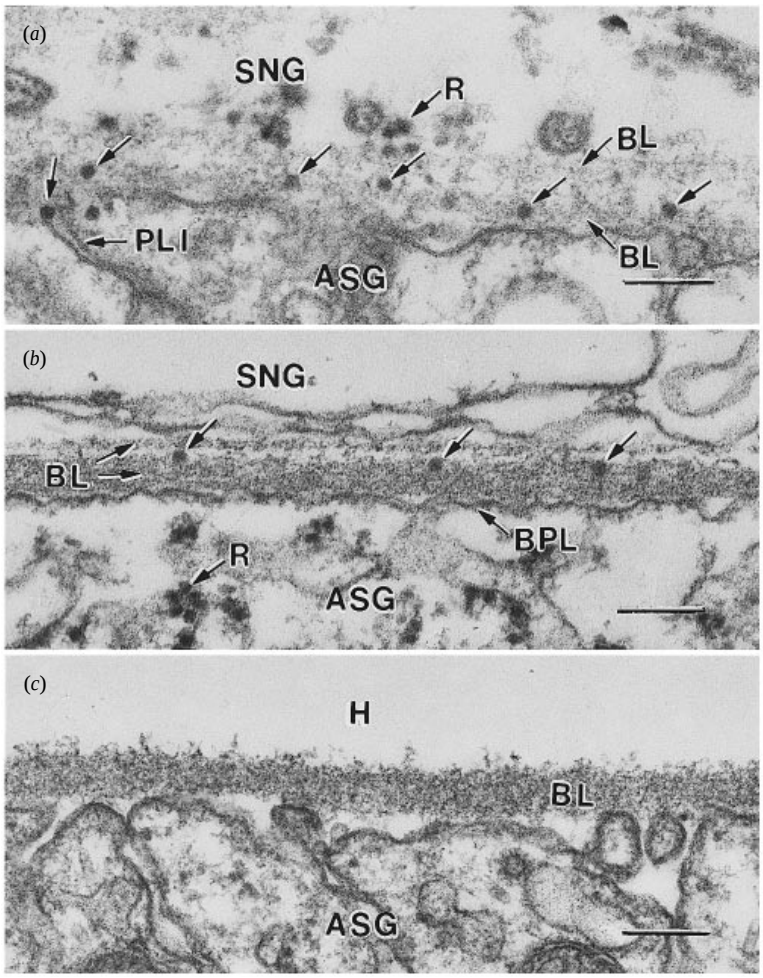

Fig. 1. Transmission electron micrographs of ASG basal laminae of three aphid species showing three types of RPV association following microinjection with 0·7 ng RPV and a 24 h inoculation feeding on oats. (*a*) Virions of RPV (unlabelled arrows) embedded in *R. padi* ASG basal lamina (BL) and in the opening of a plasmalemma invagination (PLI) following virion penetration of the basal lamina. (*b*) Virions of RPV embedded only in the outer surface of the ASG basal lamina in *M. dirhodum*. (*c*) View of basal lamina of *R. maidis* showing lack of virus attachment following injection with the same RPV preparation. In all species, virions were associated only with the ASG basal lamina and were not observed attached to basal laminae of adjacent tissues, such as the suboesophageal nerve ganglion (SNG) shown. BPL, basal plasmalemma; R, ribosomes; H, haemocoel. Bar, 150 nm.

Ferritin penetration of basal laminae

Both anionic and cationic ferritin were observed to disperse throughout the haemocoel of injected *S. avenae* and *R. maidis* and to penetrate through basal laminae of all tissues examined, including accessory and principal salivary glands, stylet retractor muscle, suboesophageal nerve ganglion, optic lobe nerve tissue and tracheal connective tissues in both species. Cationic ferritin tended to aggregate attached to the haemocoel face of the basal lamina, suggesting a net negative charge on the basal lamina surface. Cationic ferritin also penetrated the ASG basal lamina as individual ferritin particles. However, once penetrated they were observed to have accumulated at the openings of the invaginations of the basal plasmalemma (Fig. 2), indicating an accumulation of negative charge in that area. In contrast, anionic ferritin did not aggregate on the surface of the basal lamina. Anionic ferritin penetrated the ASG basal lamina as single particles and diffused into the membrane invaginations as single particles without aggregation or attachment to any specific sites. These observations indicated a lack of net positive charge associated with the surface of the ASG. The structure of the ASG basal lamina did not present a barrier to penetration by negatively (or positively) charged, 11 nm diameter ferritin particles. Neither anionic nor cationic ferritin was observed intracellularly in any tissues. This experiment was repeated with identical results.

Stability of colloidal gold *in vivo*

When colloidal gold suspensions were observed by TEM, loose aggregates of gold particles were observed covering the surface of grids. However, when undiluted, 20 nm anionic gold was injected into aphids and the haemolymph withdrawn and observed by TEM, gold was not recovered. If the gold solution was concentrated 50-fold prior to injection, aggregates consisting of 20–30 gold particles were observed in the haemocoel and intracellularly in connective tissue in three of six aphids examined. Aggregates were never observed to be associated specifically with the ASG, but were observed randomly throughout aphid haemocoels. Individual gold particles were rarely observed in the aphid haemocoel. These results suggested that aphid haemolymph caused colloidal gold to aggregate and precipitate *in vivo*. Therefore, aphid

Table 1. Transmission efficiency and observation of virions in the ASG of aphids following microinjection with purified BYDV-RPV particles and an inoculation access period on Coast Black oats

Aphid species	RPV injected (ng per aphid)	No. of aphids with virus observed in ASG*		Mean density†	Percentage transmission‡
		Basal lamina	Salivary canal		
R. padi	0·7	6	4	20	64
S. graminum	0·7	3	0	3	0
S. avenae	0·7	5	0	57	0
M. dirhodum-CU	0·7	6	0	5	0
M. dirhodum-PSU	0·7	2	0	13	0
R. maidis	0·7	0	0	0	0
R. padi	1·4	5	4	58	70
S. graminum	1·4	5	2	7	15
S. avenae	1·4	5	0	63	0
M. dirhodum-CU	1·4	3	0	20	0
M. dirhodum-PSU	1·4	5	0	27	0
R. maidis	1·4	0	0	0	0

* Six third or fourth instar aphids of each species were injected with approximately 0·02 μl of density gradient-purified RPV suspended in 0·01 M phosphate buffer. Individual aphids were placed on 1-week-old oat seedlings for 24 h, then prepared for TEM. For each aphid, a minimum of two sections was scanned for virus in the ASG basal lamina. The salivary gland was also scanned for intracellular virions in vesicles or in the canal lumen.

† Mean number of virions in 10 μm length of basal lamina.

‡ Percentage of approximately 22 aphids per treatment transmitting RPV following virus injection and a 5 day inoculation feeding individually on 1-week-old oat seedlings.

Table 2. Transmission efficiency and observation of virions in the ASG of aphids following microinjection with purified BYDV-PAV particles and an inoculation access period on Coast Black oats

Aphid species	PAV injected (ng per aphid)	No. of aphids with virus observed in ASG*		Mean density†	Percentage transmission‡
		Basal lamina	Salivary canal		
R. padi	1	5	6	1	6
S. graminum	1	5	0	0	4
S. avenae	1	3	2	< 1	5
M. dirhodum-PSU	1	3	3	2	0
R. maidis	1	0	0	0	0
R. padi	4	5	4	37	54
S. graminum	4	2	2	1	4
S. avenae	4	5	4	5	15
M. dirhodum-PSU	4	5	2	10	8
R. maidis	4	0	0	0	0

* Six third or fourth instar aphids of each species were injected with approximately 0·02 μl of density gradient-purified PAV suspended in 0·01 M phosphate buffer. Individual aphids were placed on 1-week-old oat seedlings for 24 h, then prepared for TEM. For each aphid, a minimum of two sections were scanned for virus in the ASG basal lamina. The salivary gland was also scanned for intracellular virions in vesicles or in the canal lumen.

† Mean number of virions in 10 μm length of basal lamina.

‡ Percentage of approximately 22 aphids per treatment transmitting PAV following virus injection and a 5 day inoculation feeding individually on 1-week-old oat seedlings.

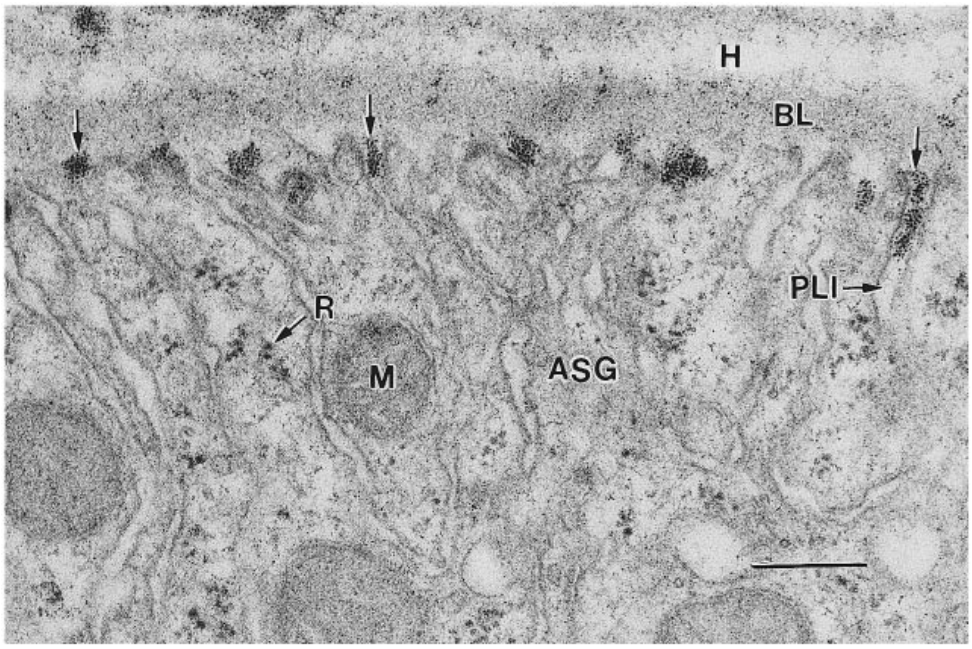

Fig. 2. Transmission electron micrograph of *S. avenae* ASG following microinjection of cationic ferritin. Positively charged cationic ferritin penetrated the basal lamina (BL) as individual particles and diffused into the lumen of plasmalemma invaginations (PLI). The accumulation of cationic ferritin at the openings of the membrane invaginations is marked by unlabelled arrows. H, haemocoel; M, mitochondria; R, ribosomes. Bar, 300 nm.

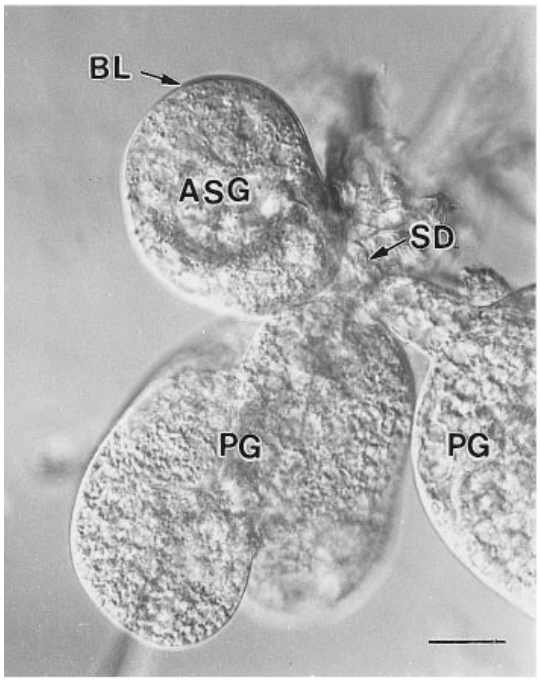

Fig. 3. Interference contrast light micrograph of a dissected salivary system from *S. avenae*. The aphid salivary system consists of two sets of a principal and accessory salivary gland (PG and ASG), respectively. The paired glands are located between the head capsule and thorax posterior to the optic lobe of the brain associated with each eye. Each pair of accessory and principal glands is connected by a common salivary duct (SD) which leads to the stylet. BL, basal lamina. Bar, 25 μm.

ASGs were dissected in order to observe the basal lamina–colloidal gold interaction *in vitro*.

Colloidal gold penetration of basal laminae *in vitro*

When the aphid salivary system was dissected, it remained intact and appeared normal by light microscopy (Fig. 3). When observed by TEM, the cytoplasm of dissected and incubated ASGs was less dense than in intact aphids, but microvilli-lined canals, ribosomes and mitochondria were present and easily identifiable. More importantly, the basal lamina appeared normal and the plasmalemma was intact.

The association of differently charged and sized gold particles with the ASG basal lamina was determined by TEM, following incubation of dissected ASGs in colloidal gold suspensions. Colloidal gold particles on the haemocoel surface of the basal lamina and only partially surrounded by basal lamina were considered to be 'attached' to the basal lamina. Particles completely surrounded by basal lamina were considered 'embedded'. Particles observed adjacent to the basal plasmalemma cell membrane were considered to have 'penetrated' the basal lamina.

When *S. avenae* ASGs were incubated in 7, 11, 14 or 20 nm anionic colloidal gold, particles of all sizes were observed outside, embedded in, and penetrating through the basal lamina of the ASG (Table 3, Fig. 4*a*, *b*). The 30 nm anionic colloidal gold was unable to penetrate the basal lamina,

Table 3. Localization of gold particles in aphid ASGs incubated in colloidal gold for 1 h

ASGs were dissected from 12 *S. avenae* and incubated at 4 °C for 1 h in colloidal gold suspensions on a rotary shaker as described in Methods. Following incubation, all glands were immediately fixed and prepared for TEM.

Gold diameter (nm)	Gold charge*	No. of aphids with gold associated with the ASG basal lamina†		
		Attached	Embedded	Penetrated
7–14	−	12	10	4
20	−	12	11	6
20	+	9	0	0
30	−	12	4	0

* Unconjugated colloidal gold has a net negative charge. Cationic colloidal gold is conjugated to poly-L-lysine giving a net positive charge.
† Gold particles observed on the haemocoel surface of the basal lamina were considered attached. Particles wholly surrounded by basal lamina and not exposed near the haemocoel surface were considered embedded in the basal lamina matrix. Gold particles located adjacent to the basal plasmalemma or in plasmalemma invaginations were considered to have penetrated through the basal lamina.

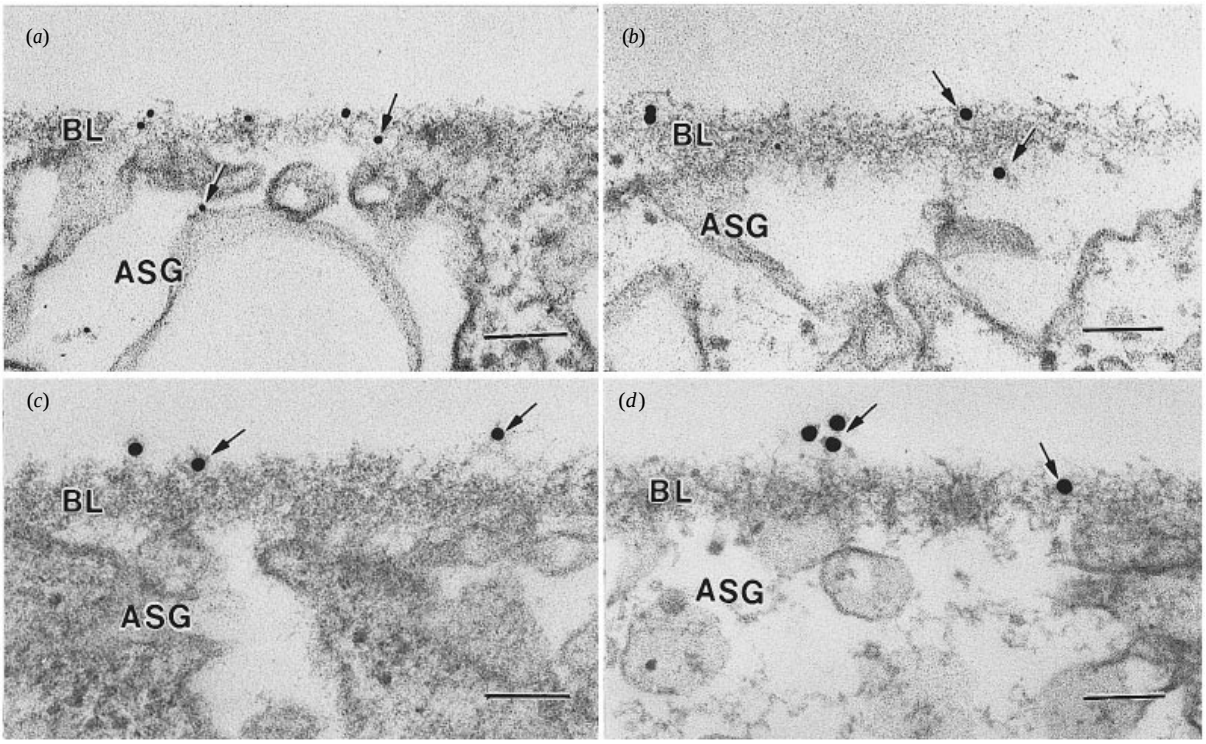

Fig. 4. Transmission electron micrograph observations of *S. avenae* ASG basal laminae (BL) following a 1 h *in vitro* incubation in anionic or cationic colloidal gold (arrows) of various diameters. (*a*) Anionic 7–14 nm diameter gold particles embedded in and having penetrated the BL. (*b*) Anionic 20 nm diameter gold particles embedded in and having penetrated the BL. (*c*) Cationic 20 nm diameter gold particles attached to the BL but not embedded or penetrated. (*d*) Anionic 30 nm diameter gold particles attached to the surface and embedded in BL near the haemocoel surface but not observed to penetrate through the BL. Bar, 150 nm.

although it was observed occasionally embedded near the outer surface of the basal lamina (Fig. 4*d*).

When dissected salivary glands were incubated in cationic 20 nm diameter gold, particles were observed attached only to the outer haemocoel surface of the basal lamina (Table 3), and were never observed penetrating basal laminae (Fig. 4*c*). No gold particles of any size or charge were observed intracellularly in the ASG or any other cell types.

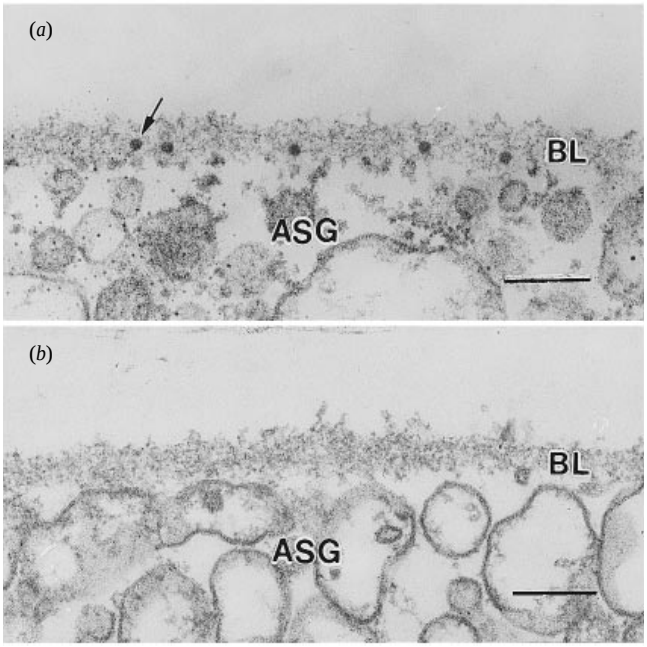

Fig. 5. Transmission electron micrograph of *S. avenae* and *R. maidis* ASG dissected and incubated for 1 h in purified MAV particles prior to rinsing and fixation for observation. (*a*) MAV particles (arrows) embedded in the basal lamina (BL) of *S. avenae*, an efficient vector of MAV. (*b*) No MAV particles were observed associated with the basal lamina of *R. maidis*, which does not transmit MAV. Bar, 150 nm.

MAV penetration of basal laminae *in vitro*

In a previous study (Gildow & Gray, 1993), MAV particles were readily observed to associate *in vivo* with the ASG basal lamina of *S. avenae*, the most efficient vector of the virus. Injected MAV was never associated with the ASG basal lamina of *R. maidis*, a non-vector. To test the specificity of luteovirus attachment and uptake by the ASG basal lamina *in vitro*, dissected salivary glands of *S. avenae* and *R. maidis* were incubated in solutions containing purified MAV. When dissected salivary glands of *S. avenae* were incubated for 1 h in purified virus suspended in 0·01 M phosphate buffer, MAV particles were consistently observed embedded at high densities only in ASG basal lamina in all three aphids examined (Fig. 5 *a*). When dissected ASGs of *R. maidis* were incubated for 1 h in the same MAV suspension, MAV particles were never observed to be associated with any of the ASGs (Fig. 5 *b*). The specific association of MAV with dissected ASGs suggested that the mechanism involved in attachment to the basal lamina was similar *in vitro* and *in vivo*.

Discussion

The basal lamina of the aphid ASG clearly plays a role in governing the transmission of luteoviruses. The regulation of virion penetration of the basal lamina appears to involve two stages: virus attachment to the basal lamina and a change in the permeability of the structure to allow virus particles to move across. The PAV and RPV isolates of BYDV were unable to associate with the ASG basal lamina of the non-vector, *R. maidis*, and no transmission was observed. Similarly, when dissected salivary glands of *R. maidis* were incubated in purified MAV, virions failed to attach to or penetrate the ASG basal laminae. These observations suggest that our biotype of *R. maidis* lacks some component of the ASG basal lamina common to the other aphid species examined. This may be one reason why *R. maidis* is a poor vector for these three BYDVisolates.

Virus attachment need not be an all-or-nothing phenomenon to have an effect on luteovirus transmission. The affinity of PAV and RPV for binding to the ASG basal lamina was different for the various aphid species. The density of PAV particles associated with the ASG basal lamina corresponded to transmission efficiency (Table 2). This observation suggests that, in addition to the selectivity that occurs on the ASG plasmalemma, transmission efficiency of an aphid species or biotype may be partially regulated by the affinity of the virus for molecules located on the surface of the ASG basal lamina.

The basal laminae of insects contain many complex glycoproteins, some of which have been shown to be receptors important for cell adhesion processes (Pedersen, 1991). Viruses may have evolved to exploit receptors located on basal laminae. Sindbis virus binds to cells via a laminin receptor located on basal laminae which is highly conserved among mammals and insects, and is thought to be partly responsible for its wide host range (Wang *et al*., 1992). No receptor has been identified on the basal lamina of the aphid ASG. However, data presented here indicate that the transmission of luteoviruses requires virions to attach to the ASG basal lamina. Furthermore, binding of all BYDVs examined was specific to the ASG basal lamina, and the viruses bound with different affinities.

Luteovirus movement through the basal lamina may require some change in the structural arrangement of the basal lamina components. The size exclusion limit of the basal lamina of the aphid ASG is between 20 and 30 nm and is charge-dependent. This is larger than the 6–15 nm range reported for other insect basal laminae (Giorgi *et al*., 1991; Houk *et al*., 1986; Reddy & Locke, 1990). It is likely that some modification of the glycoprotein lamina structure is required to facilitate transport of 25 nm diameter luteoviruses through the basal lamina. Permeability of the ASG basal lamina varied among aphid species in response to different luteoviruses. RPV and PAV had relatively high affinities for the ASG basal laminae of both biotypes of *M. dirhodum*, but the virus tended to be concentrated at the haemolymph side of the basal lamina and was very rarely observed to have penetrated. Inefficient penetration is likely to have contributed to the low transmission efficiencies for PAV.

The structure of the ASG supports a role in the uptake of macromolecules and suggests a likely target for viruses to exploit as a mechanism of escape from the aphid vector. The

plasmalemma of several insect cell types, specialized for the uptake of macromolecules from the haemolymph, is folded inward to form deep invaginations with negative charges on the membrane at the entrance ways (El Shoura, 1986; Locke, 1986). The plasmalemma of the aphid ASG is also invaginated and cationic ferritin accumulated at the openings of plasmalemma invaginations. Luteoviruses are endocytosed into the ASG cell cytoplasm specifically from the lumen of plasmalemma invaginations (Gildow & Gray, 1993). It is possible that an accumulation of negative charges at the openings of the plasmalemma invaginations may play a role in channelling luteoviruses into the lumen of the invaginations.

The data presented here verify that, to be transmitted, luteoviruses must first be recognized by, and penetrate through, the extracellular barrier of the ASG basal lamina. Depending on the specific luteovirus–aphid species combination, the basal lamina may act as an absolute barrier to transmission by preventing virus attachment, or impeding penetration, thus reducing transmission efficiency. Alternatively, in efficient vectors, the ASG basal lamina may attract and concentrate virions, thus facilitating transmission. Once the luteovirus penetrates the ASG basal lamina, the virus particles arrive at the ASG plasmalemma. The ASG plasmalemma presents a second distinct selection barrier regulating virus recognition, attachment and endocytosis into the ASG and thus transmission. The specific association of BYDV with the ASG and not with other tissues indicates unique, but as yet undetermined, characteristics of the ASG basal lamina. Additional work is required to identify the composition of the basal lamina and those components that may specifically interact with luteovirus capsid proteins.

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